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Concomitant effect of mitomycin C and gamma radiation on sarcoma 180 ascites tumour bearing mice

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Abstract: Mitomycin-C (MMC) and gamma radiation were administered in different dose combinations on 5 day old Sarcoma 180 ascites tumour bearing swiss albino mice for enhanced tumour cell kill. The MMC dose was maintained below cytotoxicity level and the radiation was also applied within tolerable range for the host. Viable tumour cell numbers with respect to untreated control reduced appreciably during combined treatments. Isobolograms were constructed from dose response curves to find out whether interaction of MMC with gamma radiation was additive or not. The population of the cells that survived the treatments were compared at their ultrastructural level. Haematological parameters of host mice and increase in their life span were determined. Analysis of the data helped to select optimum doses of MMC and gamma radiation for maximum effectivity with minimum toxicity.

Keywords: Sarcoma 180 tumour cell, mitomycin C, gamma radiation, isobolograms, ultrastructure.

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1. Introduction

In recent years, chemotherapy is used in combination with radiotherapy in both animals and human for management of neoplastic diseases applied either concomitantly or sequentially (Bellamy and Hill 1984, Majumdar and Mukherji 1987, Karuri and Mukherji 1987). Whenever cytotoxic agents are combined for the management of neoplastic diseases, the question arises: Is the effect of combination greater or less than would be expected on the basis of the effects of the agents used alone (Steel and Peckham 1979). To find an answer, Sarcoma 180 ascites tumour bearing mice were exposed to different doses of MMC and/or gamma radiation. Iso-effect plots (isobolograms) were constructed from dose response curves to analyze the interaction between MMC and radiation on tumour cells. The effect on host mice were assessed by determining their mortality rate and haematological parameters. Ultrastructure of tumour cells revealed the changes brought by combined treatment.

2. Materials and methods

Sarcoma 180 ascites tumour cells were maintained by serial intraperitoneal transplantation (10^7 cells per swiss albino mice, av. wt. 20-22 g, 8-10 wks. old). On the 5th day after tumour transplantation the mice were divided into four groups. Group A of animals was kept as control, Group B of animals was given a single i.p. injection of MMC in 0.01 M phosphate buffer (pH 7.2) in a dose of 4 mg or 7 mg per kg body weight of mouse. Group C of animals was whole body irradiated with 4 Gy or 8 Gy at a dose rate of 0.62 Gy/minutes (137 Cs source, picker USA, at Cancer Hospital, Calcutta). Group D of animals received MMC and gamma radiation concomitantly in the following sequence i.e. (i) 4 mg MMC+4 Gy, (ii) 7 mg MMC+4 Gy, (iii) 4 mg MMC+8 Gy (iv) 7 mg MMC+8 Gy. The viable number of tumour cells per ml of ascitic fluid in both untreated and treated groups were measured in different days using trypan blue dye exclusion test. The experiment was repeated for 5 times with 10 mice in each group. Mortality rate R of treated or untreated host mice was determined from the relation $R = -\frac{dN/N}{dt}$ (Andrews 1974) where N is the number of mice at time t. In the present work unit of time was choosen to be two days. Haematological parameters such as haemoglobin concentration, RBC and WBC count and bone marrow cellularity were measured according to standard procedure (Kolmer *et al* 1969). For electron microscopy tumour cells from treated mice were fixed in 1.5% paraformaldehyde : 1% Glutaraldehyde, post fixed in 1% osmium, enbloc with 0.5% uranylacetate and dehydrated in ethanol and embedding was done in epoxy resin (Spurr 1969). Ultrathin sections were stained in lead citrate and examined with Hitachi H-600 transmission electron microscope operated at 75 kv.

3. Results

When mitomycin C and gamma radiation were administered either singly or in different dose combinations on 5 day old Sarcoma 180 swiss albino mice (as described in materials and methods), the number of viable tumour cells with respect to untreated control reduced appreciably with days (Majumdar and Mukherji 1987). The fraction of tumour cells that survived any treatment on any day was determined from the expression $SF = T/C$ where C is the viable number of untreated cells and T is the viable number of cells on a particular day after treatment.

For isobologram analysis the cell survival data obtained on 12 day after transplantation were used and they were fitted through least square analysis in three different mathematical models depending on the mode of treatment (MMC, radiation and MMC plus radiation) (Alper 1980, Deen and Williams 1979). Table 1 shows the expression used for different treatment modalities and constants determined from experimental data. Using these constants, complete dose response curves were determined upto level of 15% survival as shown in Figures 1

Table I. Expressions used to fit cell survival data.

Treatment modalities	Expressions used	Experimentally determined constants
Radiation	$\ln SF = -(\alpha D + \beta D^2)$	$\alpha = 0.047$ Gy $^{-1}$ $\beta = 5.687 \times 10^{-4}$ Gy^{-2}
Mitomycin C	$\ln SF = \ln A - KC^2$	$A = -0.565$ $K = 2.18 \times 10^{-3}$
Mitomycin C Plus Radiation	$\ln SF = \ln A' - b.D$	For 4 mg MMC $A' = 0.680$ $b = 0.140$ For 7 mg MMC $A' = 0.543$ $b = 0.120$

and 2, Figure 1 for single treatments and Figure 2 for combined treatments. Isobolograms (Figure 3) were constructed for two levels of cell survival (IE_{40} and IE_{20})

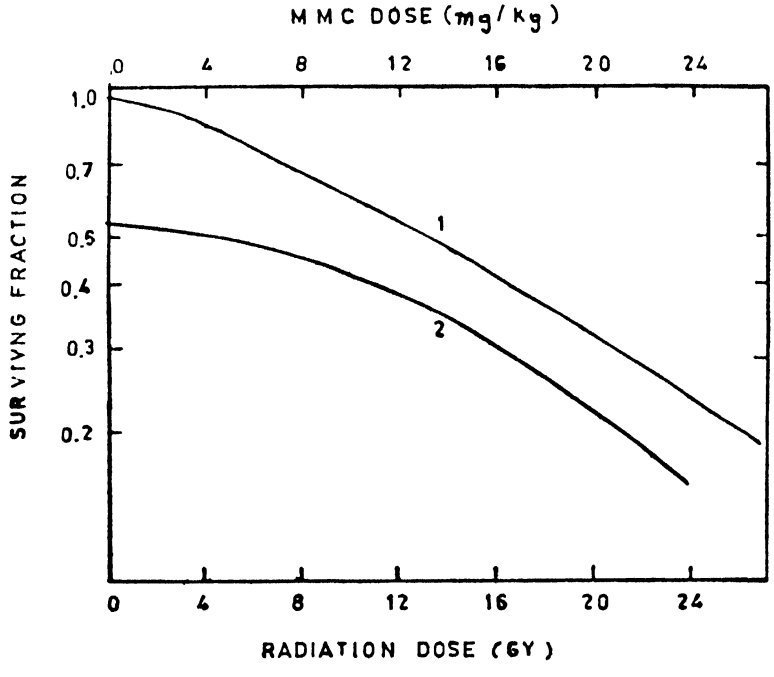

Figure 1. Dose response curves for single treatment (1) Radiation, (2) Mitomycin C.

from Figure 1 following the methods of Steel and Peckham. According to the concept of additivity introduced by these authors, if the experimentally determined

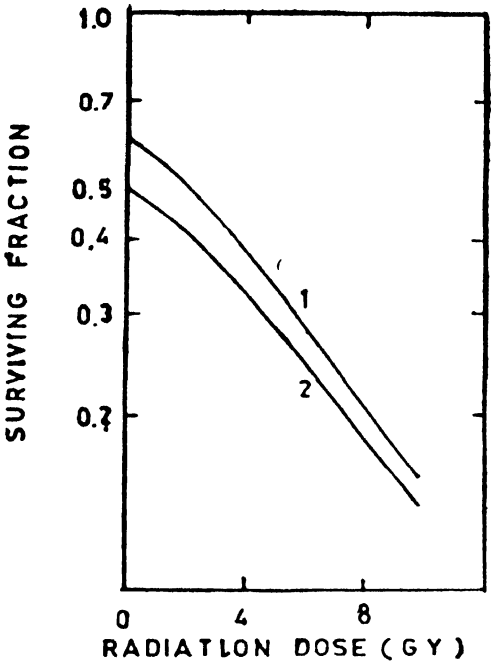

Figure 2. Dose response curves for combined treatment (1) 4 mg MMC plus radiation, (2) 7 mg MMC plus radiation.

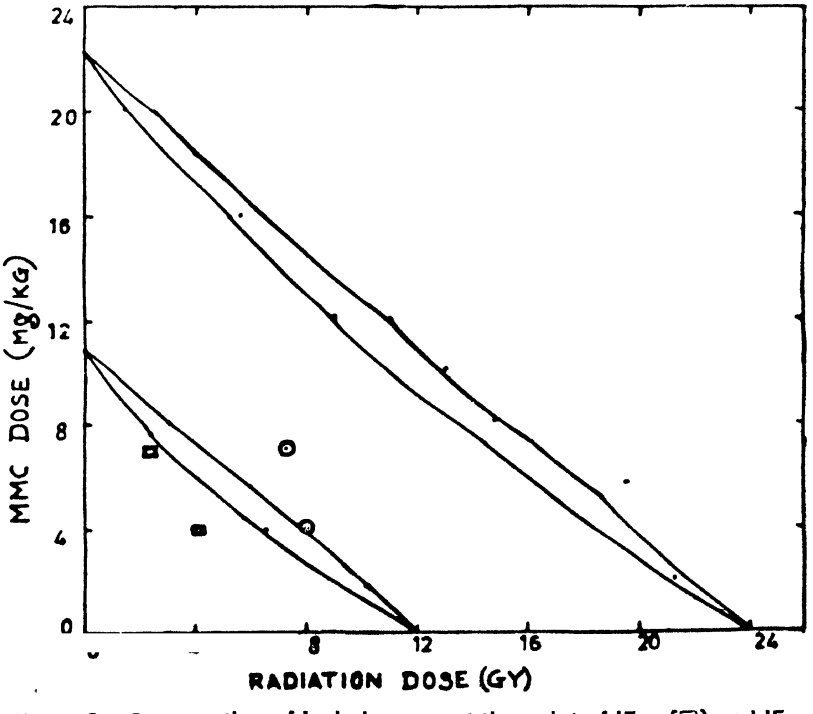

Figure 3. Construction of isobolograms at the point of IE_{40} (□) and IE_{20} (○).

data fell inside the envelope of additivity this would mean that interaction between two agents was "additive". On the other hand if it fell left to envelope of additivity this would mean interaction between the same agents was "supra additive". It is apparent from Figure 2 IE_{40} were produced by the combination of 4 mg MMC+3.6 Gy and 7 mg MMC+2.6 Gy respectively and similarly IE_{80} were produced by the combinations of 4 mg MMC+8 Gy and 7 mg MMC+7 Gy respectively. The location of data points all fell to the left of their respective envelope of additivity (Figure 3). The dose combinations used in the present work as mentioned in materials and methods, are almost similar to the dose combinations which produced IE_{40} and IE_{80} effects. Hence it is suggested that all the combinations used in the present work produced "supra additive" interaction in tumour cell kill.

Mortality rate of host mice undergoing different treatments are shown in Figure 4 together with that of the untreated group. The slope of mortality curve

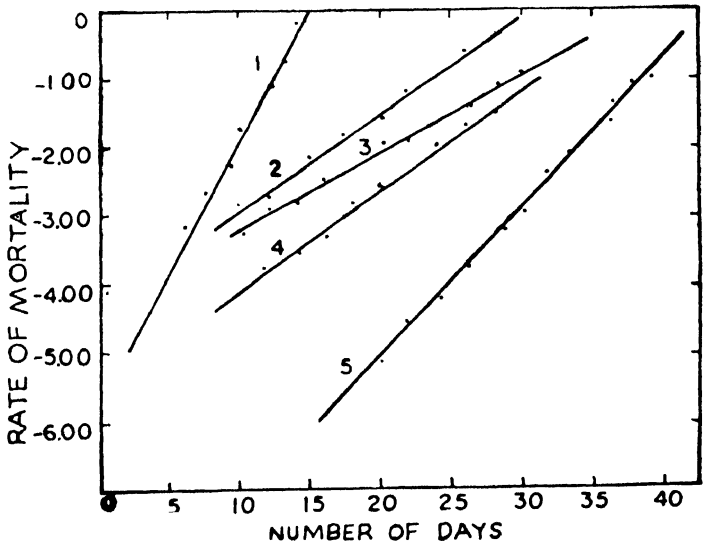

Figure 4. Mortality plot for Sarcoma 180 tumour bearing host mice (1) 7 mg MMC+8 Gy ; (2) 8 Gy ; (3) untreated control ; (4) 4 Gy ; (5) 4 mg MMC +4 Gy. The ordinate is in natural log scale.

increased in case of all treatments. However, life span increased appreciably with respect to untreated control in case of 4 Gy and 4 mg MMC+4 Gy respectively. (data not shown)

Table 2 shows the haematological changes of few treated and untreated group of mice on 12th day after transplantation. With respect to untreated control and 7 mg MMC treated group, there is a sharp decrease in haemoglobin concentration, WBC count and bone marrow cellularity in case of 7 mg MMC+8 Gy treatment.

This observation explain the increase in mortality rate in case of 7 mg MMC+8 Gy treatment which caused toxicity development in the host mice.

The changes brought at the ultrastructural level due to combined treatment (4 mg MMC+8 Gy) is shown in Figure 5 ; broken microvilli, swollen mitochondria,

Figure 5. Sarcoma 180 tumour cells after 5 days of *in vivo* treatment with 4 mg MMC + 8 Gy, 8400. Electron micrograph showing broken microvilli (arrow heads), swollen mitochondria (M), intercellular spaces (IS), segregated nucleolous into two distinct zones, light (L) and dark (D), increased number of perichromatin granules (arrows).

increase of intercellular spaces, fragmented nucleus with condensed heterochromatin and segregated nucleolous indicated the degenerating condition of a tumour cell (Ghadially 1985).

Table 2. Effect of mitomycin C and gamma radiation on haematological changes in Sarcoma 180 tumour bearing mice (after 12 days of transplantation). Mean ± *S. E., No.* of experiments-5, with 5 mice in each group.

	Untreated control	7 mg MMC	8 Gy Radiation	7 mg MMC +8 Gy
Haemoglobin (g/dl)	9.10 ± 0.45	9.20 ± 0.85	8.95 ± 0.15	7.80 ± 0.60
RBC ($\times 10^6/\mu l$)	4.35 ± 0.52	4.30 ± 0.21	4.32 ± 0.35[a]	3.55 ± 0.75
WBC ($\times 10^3/\mu l$)	7.90 ± 0.61	7.75 ± 0.24[a]	7.95 ± 0.20[a]	3.28 ± 0.48
Nucleated cells/Femur ($\times 10^6$/ml)	15.60 ± 0.27	16.50 ± 0.75[a]	15.45 ± 0.65[a]	7.20 ± 0.15

In comparison with control (a) P · 0.5, in all other cases P 0.05.

4. Conclusion

Concomitant administration of Mitomycin C and of gamma radiation on Sarcoma 180 ascites tumour bearing mice produced supra additive cell kill at different

survival level. Survivality of host mice increased in case of MMC+4 Gy treatment and MMC+8 Gy of radiation developed toxicity.

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